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SEED PRODUCTION, VIABILITY, AND DORMANCY IN THE NUTGRASSES CYPERUS ROTUNDUS AND C. ESCULENTUS [1]

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INTRODUCTION

Although nutgrass (*Cyperus rotundus* L.) is one of the most noxious weeds in the southern part of the United States a search of the literature reveals no published data on seed production or seed germination in this country and only one record from other countries. There are many published but controversial statements relating the importance of seed production and viability to the reproduction of nutgrass. Except for the work reported by Ranade and Burns (*13*) [3] these statements are not supported by data.

Among those who have attached importance to the reproduction of nutgrass by seeds are Davis and Hawkins (*3*), Dewey (*4*), Georgia (*6, pp. 68–69*), Godfrey (*7*), Henke (*9*), Muenscher (*11, pp. 174–176*), Pillay (*12*), Schultz (*17*), the Extension Agronomy Department of the Virginia Agricultural and Mechanical College and Polytechnic Institute, (*19, pp. 49*), and Saunders (*15*) who referred to it as "uintjies" or "watergrass." According to Andrews (*1*), Du Preez (*5*), Ranade and Burns (*13*), and Robbins, Crafts, and Raynor (*14, pp. 468–469*), reproduction by seeds is of only minor importance. Hansen,[4] Holcomb,[5] MacMahon (*10*), and Smith and Fick (*18*) reported that they were unable to find viable seeds of nutgrass.

Holcomb [5] made histological studies of the possible causes of sterility in nutgrass grown in Alabama. She stated that this species has never been known to produce viable seeds in the United States, but presented no results or references to substantiate this statement. She concluded that possible causes of sterility were the failure of pollen to germinate and early disintegration of megagametophyte tissue.

[1] Received for publication October 2, 1945. This investigation was begun when both authors were employed by the Alabama Department of Agriculture and Industries and was completed by the senior author in his present position.

[2] The authors wish to express their appreciation to Drs. W. C. Muenscher and G. H. M. Lawrence, Cornell University, and to Dr. G. P. Steinbauer, University of Maine, for supplying seeds of *Cyperus esculentus*.

[3] Italic numbers in parentheses refer to Literature Cited, p. 318.

[4] H[ANSEN], A. A. NUTGRASS OR COCO (CYPERUS ROTUNDUS). U. S. Bur. Plant Indus. 2 pp. 1921. [Processed.]

[5] HOLCOMB, V. POSSIBLE CAUSES OF STERILITY IN CYPERUS ROTUNDUS L. HISTOLOGICAL STUDIES. 1940. [Unpublished master's thesis. Copy on file Ala. Polytech. Inst., Auburn.]

Ranade and Burns (*13*) tested, in sand in Petri dishes at about 29° C., seeds of nutgrass grown locally in India. A germination of 72 percent was obtained from seeds which had previously been heated for 3 hours at 51°, whereas seeds in control tests germinated from 20 to 30 percent. It was noted that germination increased with time of storage up to 4 months. In one series of tests a germination of 80 percent was obtained from untreated seeds which had been stored for 4 months; however, a sample of untreated freshly harvested seeds germinated only 24 percent. These tests are open to criticism because only 25 seeds were used in each test. The authors obtained an average of only 1.5 percent germination from 22,086 seeds which were sown in pots placed in the open on a veranda.

Although yellow nutgrass (*Cyperus esculentus* L.) is a serious weed pest in the northeastern and northwestern parts of the United States and other areas where potatoes are grown abundantly, the authors have found no reference to work having been done on the germination of seeds of this species.

Because of the paucity of data on seed production and viability in the nutgrasses growing in the United States, achenes were collected over a period of 3 years, stored under various conditions, and tested for germination. The results of these tests are reported in this paper.

STRUCTURE OF THE SEED OF CYPERUS

The fruit or achene in *Cyperus* is usually referred to as a seed, and in this paper the term "seed" is used when referring to achenes. The trigonous achenes of *Cyperus rotundus* and *C. esculentus* are approximately 1.5 mm. by 0.8 mm. in size; the seeds are about 1.2 mm. by 0.5 mm., and the embryos 100μ by 300μ. Structure of seeds and some stages of embryo and seedling development in nutgrass and yellow nutgrass are illustrated in figure 1.

A hard and relatively thick pericarp (fig. 1, *A*, and *F*) encloses a thin seed coat which has been reduced to a few cells in thickness. The endosperm is differentiated into starchy endosperm and a peripheral oil layer. The embryo is microscopic in size, lies at the base of the seed, and is in contact with the starchy endosperm and oil layer; it appears globular to ovate, and high magnification is required to show the differentiation of its parts. During germination the apical end of the embryo elongates into the sucker, or absorbing organ, through which food passes into the seedling. The basal end of the embryo passes through the loosely arranged cells at the base of the seed during germination. An enlargement occurs outside the seed coat from which the primary root and shoot appear.

Goebel (*8, pp. 413–414*) described and illustrated the embryo and two stages of seedling development in *Cyperus alternifolius*. Schneider (*16*) made a careful morphological study of the entire seed and observed the development of the embryo in the same species. She showed that the embryo, although microscopic in size, is differentiated into coleoptile, root, shoot, and haustorium. Boyd (*2, pp. 170–177*), working with *Cyperus alternifolius* and *C. natalensis*, confirmed the work of Schneider but disagreed with the latter's interpretation of the origin and development of the cotyledon.

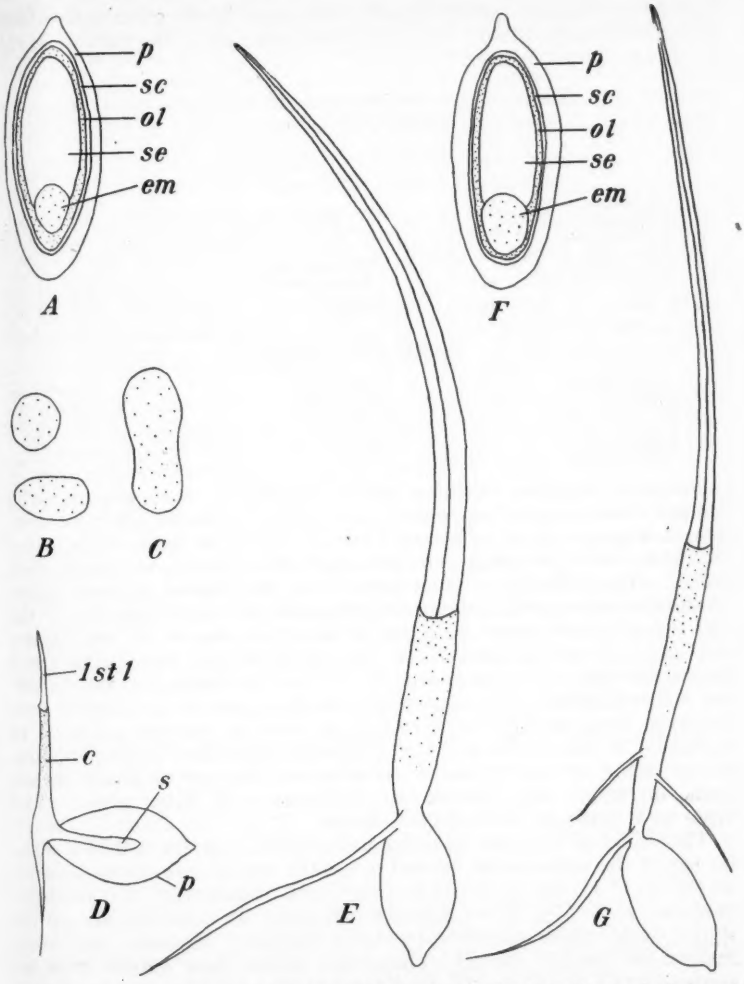

FIGURE 1.—*Cyperus rotundus: A*, Median longitudinal section of dormant achene; *B*, Dormant embryos; *C*, germinating embryo; *D* early stage of seedling development; *E*, later stage of seedling development. *Cyperus esculentus: F*, Median longitudinal section of dormant achene; *G*, State in seedling development comparable with *E*. *A* and *F: p*, pericarp; *sc*, seed coat; *ol*, oil layer; *se*, starchy endosperm; *em*, embryo. *D: 1st l*, first leaf; *c*, coleoptile; *s*, sucker; and *p*, pericarp.

MATERIALS AND METHODS

All seeds were harvested and threshed by hand. The inert material and foreign seeds were removed by hand and by an air-blast seed separator. Each sample was separated into four fractions based upon the specific gravity of the seeds. Only the seed fractions of each lot

having the highest specific gravity were used in the germination tests unless otherwise noted. Pertinent data relating to the harvest of the seeds are shown in table 1.

TABLE 1.—*Location and date of harvest of seed lots*

Lot	Place of harvest	Habitat	Date of harvest
	CYPERUS ROTUNDUS		
1	Shorter, Ala	Cornfield	Sept. 6, 1941.
4	Montgomery, Ala	do	Sept. 13, 1941.
10	Wetumpka, Ala	do	Sept. 20, 1941.
15	Elmore, Ala	do	Oct. 4, 1941.
20	Montgomery, Ala	Velvetbean field	Oct. 24, 1941.
22	do	Soybean field	Oct. 14, 1942.
23	do	Cornfield	Oct. 19, 1942.
24	do	Garden	Nov. 7, 1942.
31	do	do	Nov. 13, 1943.
	CYPERUS ESCULENTUS		
12	Wetumpka, Ala	Cotton field	Sept. 20, 1941.
17	Ithaca, N. Y	Not known	Sept. 27, 1941.
25	Wetumpka, Ala	Cotton field	Oct. 19, 1942.
26	Ithaca, N. Y	Not known	Oct. 1942.
32	Orono, Maine	Greenhouse	Aug. 1944.
33	Fryeburg, Maine	Field	Aug. 25, 1944.

Seeds of *Cyperus rotundus* and *C. esculentus* were placed under storage and germination conditions within 6 weeks after harvest. For storage, seeds were placed between layers of moist filter paper in Petri dishes overlaid and underlaid with cotton saturated with water. The following temperatures were maintained in germination chambers and modified domestic refrigerators for storage: (a) 2° C., (b) 10°, (c) alternations of 2° for 16 hours per day to 20° for 8 hours per day, (d) alternations of 10° for 16 hours per day to 20° for 8 hours per day, (e) alternations of 20° for 16 hours per day to 30° for 8 hours per day, (f) air-dry at room temperature. Samples were removed from storage for germination tests at various intervals as indicated in the tables and text. The germination substratum consisted either of two disks of germination blotters or moist cotton pads, on which were placed two thicknesses of filter paper. The tests were made in covered Petri dishes.

The seeds of *Cyperus rotundus* were sewed, usually 200 in a dish, on top of the substratum, placed in a dark seed germination chamber at 20° ±1° C. for 16 hours per day, and transferred to a daylight chamber at 35° ±1° for 8 hours per day. In some of the earlier initial tests room temperature with daylight exposure was used instead of the 35° daylight chamber. In the first earlier tests on seeds of lots 1, 4, 10, and 15, both tap water and a 0.2-percent solution of potassium nitrate were used for moistening the substratum, but in all the later tests only the potassium nitrate solution was used. The duration of most tests ranged from 6 to 8 weeks, but many were prolonged to 11 and 14 weeks, some to 18 weeks, and 7 tests to 8 months. In a few preliminary tests only 25 to 50 seeds per test were used, but most tests reported are based upon duplicates of either 100 or 200 seeds. Normal and abnormal seedlings were recorded separately. Normal seedlings were those which developed a coleoptile, first leaf, and root; abnormal seedlings developed a green or chlorotic coleoptile with or without first leaf, but without a root. Possibly the failure of

seedlings to develop roots in some instances was due to the effects of the potassium nitrate. Unless otherwise indicated the germination reported is the total of normal and abnormal seedlings.

The results reported for seeds of *Cyperus esculentus* are based upon 400 seeds per test. These were tested at an alternating temperature of 20° C., in darkness, to 30° in daylight, usually for 42 days.

EXPERIMENTAL RESULTS

SEED PRODUCTION IN CYPERUS ROTUNDUS

At first, difficulty was experienced in locating infestations of nutgrass which produced mature seeds. After considerable experience, it was found that infestations in fields which had been cultivated only during the spring months were more likely to bear mature seeds than infestations among perennial grasses, or in crops which had been cultivated until late June or July. It was observed that plants occurring in dry upland fields of corn did not produce mature seeds, but that seeds were matured in cornfields located in moist river valleys. Mature seeds were also found on infestations in spring gardens where cultivation had been discontinued in May.

The number of inflorescences comprising the three lots harvested in 1942 was determined before threshing. Because of the difficulty in locating the limits of individual plants the determinations were based upon the number of inflorescences. The seeds were hand-threshed in a manner that would insure the removal of all the seeds from the inflorescences with minimum loss. The light, immature seeds and chaff were separated from the heavy seeds by a seed blower. After the immature seeds had been removed the heavy portion was separated into four fractions based upon specific gravity. Germination tests were made at many intervals on seeds of the two heavier fractions, and at two intervals on seeds of the two lighter fractions. The results of some of these determinations are set out in table 2.

TABLE 2.—*Seed production and viability of 4 collections of Cyperus rotundus inflorescences*

Lot number and number of inflorescences	Magnitude of blowing [1]	Seeds threshed	Average seeds per inflorescence	Total weight of seeds	Average weight per seed	Average germination	Seeds tested	Maximum germination of heavy seeds
		Number	*Number*	*Milligrams*	*Milligrams*	*Percent*	*Number*	
Lot 22:								
586 inflorescences	B–40	888	1.515	192.2	0.216	1.3	200	17 percent; 100 seeds heated at 59° C.
	B–45	99	.169	10.3	.104	0	200	
	B–50	1,223	2.087	200.8	.164	0	800	
	R–50	1,461	2.493	442.5	.303	7.2	1,236	
Lot 23:								
511 inflorescences	B–40	198	.387	27.3	.138	0	200	9 percent; 100 seeds heated at 59° C.
	B–45	452	.885	59.2	.131	0	200	
	B–50	258	.505	42.5	.165	0	400	
	R–50	759	1.505	215.5	.280	3.2	765	
Lot 24:								
1,454 inflorescences	B–40	1,512	1.040	271.2	.179	0	200	9 percent; 100 seeds heated at 59° C.
	B–45	2,073	1.426	279.0	.135	0	200	
	B–50	1,919	1.320	342.0	.178	0	400	
	R–50	3,079	2.118	901.9	.293	2.3	2,668	
Lot 31:								
Number of inflorescences not determined	B–45	[2]	[2]	[2]	[2]	0	800	9 percent; 400 seeds heated at 60° C.
	B–50					0	800	
	B–55					5.1	800	
	R–55					7.0	300	

[1] B—Fraction removed by blowing at the various valve openings; R—residue at the maximum dial opening of the seed blower.

[2] Not determined.

The germination data shown in table 2 indicate that the three lighter fractions can be disregarded in determining seedling producing capacity. This was confirmed by other tests in which seeds of all fractions were dissected. The two lighter fractions were found to consist of empty seeds (pericarp and seed coat without contents). The second heaviest fraction consisted of seeds with a small amount of endosperm tissue but usually without embryos.

SEED PRODUCTION IN CYPERUS ESCULENTUS

One infestation of yellow nutgrass was found by the authors in Alabama. No attempt was made to determine the rate of occurrence of mature seeds, but only a few viable seeds were obtained from several inflorescences. This same condition was found to prevail when the area was visited the second year.[6]

Lot 32 was harvested from greenhouse plantings at Orono, Maine, and lot 33 from a field at Fryeburg, Maine, in August 1944. The seeds from 100 inflorescences of lot 32 and 25 inflorescences of lot 33 were separated into four fractions with an air-blast seed separator. The number of seeds and respective percentages of germination for each of the four fractions of lot 32 are as follows: First fraction (lightest)—5,225 seeds, 2.5 percent germination; second fraction—2,675 seeds, 34 percent germination; third fraction—1,700 seeds, 59 percent germination; fouth fraction (heaviest)—825 seeds, 62 percent germination. Corresponding values for lot 33 are as follows: First fraction—1,130 seeds, 20 percent germination; second fraction—21,441 seeds, 58 percent germination; third fraction—23,663 seeds, 91 percent germination; fourth fraction—4,026 seeds, 95 percent germination. This is an average of 75.6 percent germination for the lot and the equivalent of 1,521 seedlings per inflorescence. The seeds of lot 32 were stored air-dry at room temperatures for 2½ months and those of lot 33 for 4½ months before being tested in daylight at 20° to 30° C. alternating temperatures for 6 weeks on blotters moistened with a 0.2-percent solution of potassium nitrate.

VIABILITY AND DORMANCY IN SEEDS OF CYPERUS ROTUNDUS

Immediately after harvest 200 seeds each of lots 1 and 4 were planted in sterile soil and placed on a greenhouse bench. None of these seeds germinated during a test period of 3 months. Samples of 100 seeds of lots 1, 4, 10, and 15 were placed in germinators at 20° to 30° C. alternating temperatures in the laboratory immediately after harvest. The first sprouts emerged in these tests 4 months later. Germination gradually increased until the tests were discontinued at the end of 13 months when the total germinations were as follows: Lot 1—15 percent; lot 4—5 percent; lot 10—2 percent; lot 15—3 percent.

The five lots of seeds harvested in 1941 were stored under the six temperature and moisture conditions previously mentioned. Seeds of the lots harvested in 1942 were stored only at 10° C. and at room temperature as tests on the 1941 lots indicated that these were the

[6] W. C. Muenscher (private communication dated August 17, 1944) found the production of mature seeds of this species rather irregular in New York State. He stated that in some fields seeds were produced in considerable abundance, whereas in others few seeds seemed to be produced.

best storage temperatures. Lot 31, harvested in 1943, was stored only dry at room temperature. Samples were removed from these storage conditions and tested at various intervals.

Because of the low percentages of germination obtained, lack of uniformity in results, and small numbers of seeds used in some tests, the results of individual tests are not reported. The average percentages of normal and abnormal seedlings obtained for each seed lot in all tests are reported in table 3.

TABLE 3.—*Mean results of all germination tests for each lot of seed of Cyperus rotundus tested*

Item	Seed lot No.—							
	1	4	10	15	22	23	24	31
Seeds tested	*Number* 1,200	*Number* 905	*Number* 1,900	*Number* 2,450	*Number* 1,375	*Number* 825	*Number* 3,000	*Number* 2,100
Normal seedlings	*Percent* 4.66	*Percent* 7.84	*Percent* 1.79	*Percent* 1.67	*Percent* 3.83	*Percent* 2.42	*Percent* 2.06	*Percent* 1.63
Abnormal seedlings	2.75	3.09	1.53	.73	3.12	1.57	.83	2.37

The percentages of normal seedlings ranged from 1.63 in lot 31 to 7.84 in lot 4. Although seeds of different lots were not all stored or tested under identical conditions the data given in table 3 indicate the relative quality of seeds of the various lots as accurately as could be obtained from an analysis of individual tests. Little can be said about the viability of lot 31 as the seeds were stored only dry at room temperature and the greatest period of storage was 9 months. Most of the germination tests on seeds of this lot were continued for 8 months. In these tests germination began during the fourth week and the last seedlings appeared during the twenty-third week, with most of the seedlings appearing between the seventh and thirteenth weeks. The two plants shown in figure 2 developed from seedlings obtained in a laboratory test on this lot of seeds.

In table 4 the results of tests made within certain periods are averaged for each seed lot, under each storage condition. In weighing these data, one should consider that the values are averages of results obtained by testing, under different conditions, variable numbers of seeds from lots differing in viability.

Considering germination as the total of normal and abnormal seedlings, there was an apparent increase in the germination of seeds which had been stored at room temperature from 1.8 percent after 2 to 4 months of storage to 6 percent after 26 to 28 months of storage. The germination of seeds which had been stored at 10° C. increased from 1.9 percent after 2 to 4 months of storage to 7 percent after 26 to 28 months, with minor exceptions. Seeds which had been stored at 2° showed no consistent increase in germination with time of storage, but maximum germination was obtained after 8 to 16 months. The average percentages of germination obtained from seeds stored at these three temperatures were as follows: (a) Room temperature, 2.47 percent; (b) 10°, 5.39 percent; (c) 2°, 1.95 percent. There were not enough seeds stored at the alternating temperatures of 10° to 20° and 2° to 20° to justify conclusions.

FIGURE 2.—Plants of *Cyperus rotundus* grown from seeds of lot 31. Seeds germinated in laboratory chambers and seedlings subsequently transplanted into soil and grown in a greenhouse.

Ranade and Burns (*13*) reported a considerable increase in the germination of nutgrass seed which they had heated in an oven at 59° C. for 1 hour, or at 51° for 3 hours, as compared with a control sample not so treated. To determine whether such treatment would affect the germination of the lots of seed used in the present investigation 100 seeds each of lots 22, 23, and 24 which had been stored air-dry at room temperature for 84, 77, and 58 days, respectively, were heated for 3 hours at (a) 50° to 55°, (b) 59° to 60°, and (c) 59° to 65°. The treated seeds were tested for germination along with untreated sam-

TABLE 4.—*Means of results from germination tests on seeds of Cyperus rotundus, showing the effects of time and temperature of storage upon germination*

Storage temperatures	Classification of seedlings, number of seeds, and seed lots tested	Months stored									Averages and totals
		2.5–4.5	5.5–7.0	8.0–10.5	11.0–12.0	13.0–14.0	15.0–16.0	17.5–19.5	22.5–24.0	26.5–28.0	
Room temperature	Normal percent	1.3	0.0	1.5	[2]	1.6	[2]	0.0	[2]	5.0	1.33
	Abnormal do	.5	.0	1.8	[2]	1.6	[2]	0	[2]	1.0	1.14
	Seeds number	665	285	1,940	[2]	450	[2]	600	[2]	200	4,140
	Lots do	5	2	2	[2]	2	[2]	2	[2]	1	
10° C	Normal percent	1.3	0	1.8	2.0	2.9	6.6	5.3	4.3	3.0	2.98
	Abnormal do	.6	0	1.0	22.0	2.0	4.0	.4	3.4	4.0	2.41
	Seeds number	1,100	50	825	50	450	350	225	950	400	4,400
	Lots do	5	1	4	1	3	4	3	3	1	
10°–20° C. alternating	Normal percent	0	1.3	12.0	2.0	[2]	7.0	10.0	17.9	[2]	8.32
	Abnormal do	0	0	8.0	0	[2]	7.0	2.0	5.7	[2]	3.36
	Seeds number	75	75	25	50	[2]	100	100	140	[2]	565
	Lots do	1	1	1	1	[2]	1	1	1	[2]	
2° C	Normal percent	0	0	9.3	[2]	0	5.0	1.3	.4	[1] .5	1.41
	Abnormal do	0	0	6.7	[2]	0	1.3	.7	.2	0	.54
	Seeds number	125	50	75	[2]	50	240	150	550	600	1,840
	Lots do	1	1	1	[2]	1	2	2	2	2	
2°–20° C. alternating	Normal percent	0	2.7	8.0	0	[2]	6.0	3.0	5.0	[2]	3.89
	Abnormal do	0	0	4.0	0	[2]	8.0	2.0	0	[2]	2.04
	Seeds number	50	75	25	50	[2]	100	100	140	[2]	540
	Lots do	1	1	1	1	[2]	1	1	1	[2]	
Averages and totals	Normal percent	1.14	.56	1.94	1.33	2.10	6.08	2.30	4.27	2.08	2.44
	Abnormal do	.50	0	1.76	7.33	1.68	4.05	1.19	2.30	1.50	1.68
	Seeds number	2,015	535	2,890	150	950	790	1,175	1,780	1,200	11,485

[1] No seedlings were obtained from 287 seeds which had been stored at 2° C. for 35 months.
[2] No tests made.

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ples. In addition, 700 seeds of lot 31, which had been stored air-dry at room temperature for 9 months, were heated for 1 hour at 60°. These treated seeds and 400 seeds not so treated were tested for germination over a period of 8 months, The results of all these tests are shown in table 5.

TABLE 5.—*Effect of heating seeds of Cyperus rotundus at 3 temperature ranges for 3 hours upon their germination, as compared with seeds not so treated*

Lot No.	Seeds heated—						Seeds not heated	
	50° 55° C.		59° 60° C.		59° 65° C.			
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
22	6	2	11	6	3	10	7	4
23	6	0	7	2	1	0	2	4
24	0	1	3	6	3	0	0	2
31	----	----	[1] 5	4	----	----	0	1

[1] Heated at 60° C. for 1 hour.

If the totals of normal and abnormal seedlings of the four lots are averaged, the results obtained show that seeds heated at 50° to 55° C., 59° to 65°, and seeds not heated are practically the same, whereas heat treatment at 59° to 60° resulted in approximately 100 percent increase in germination.

Some observations indicated that nutgrass seeds which had failed to germinate in previous tests might yield seedlings if exposed to germination conditions a second time after a period of storage subsequent to the original tests. With this in mind, 49 such samples, representing seeds which had been stored and tested under various conditions and some that had been treated with sulfuric acid were, tested a second time. Some of the samples had been tested about 2 years earlier, some only 1 month earlier, and others ranged between these extremes. In these second germination tests the total of normal and abnormal seedlings varied by seed lots from 0.7 percent for lot 15 to 5.6 percent for lot 24. In the original tests, lot 24 gave the lowest germination of all lots tested; in the second tests it gave the highest. A total of 5,000 seeds was tested the second time with an average of 2.5 percent normal seedlings and 0.4 percent abnormal seedlings. The highest germination obtained in individual tests was 7.0, 9.0, 11.0, 12.5, and 13.0 percent. An average of 2.2 percent normal seedlings and 2.5 percent abnormal seedlings was obtained in the original tests from 3,550 seeds of which previous storage and germination records could be determined. The average germination in the second tests from the same samples was 1.9 percent normal and 1.0 percent abnormal seedlings.

In another similar test seeds of lots 10 and 15 which had been previously tested and allowed to dry at room temperature for at least 20 months were tested for germination a second time. In these tests only firm seeds which showed no evidence of infection by fungi were used. Of 362 seeds thus tested, 20 percent produced normal seedlings and 3 percent produced abnormal seedlings.

Seeds of lots 10, 15, 22, 24, and 31 which had been stored moist at 10° C., moist at 2°, and dry at room temperature, were tested as indicated below. The seeds were placed in Petri dishes on moist cotton substratum. Instead of being placed under the usual germination conditions immediately, the seeds were exposed to daylight at room temperature with the lids of the Petri dishes removed. The substratum was moistened approximately every tenth day for a month, the substratum being allowed to dry out between applications of water. At the end of this month the seeds were exposed to the usual germination conditions. In these tests an average of 3 percent normal seedlings was obtained from 1,450 seeds. A maximum germination of 17 percent from 250 seeds was observed in tests of lot 15, which had previously been stored air-dry at room temperature for approximately 29 months. Only 1 percent germination was obtained from a sample of the same lot which had been stored at 10°. Seeds of lots 22 and 24 responded differently in that approximately 6 percent of the seeds stored moist at 10° and 2° germinated whereas none which had been stored dry at room temperature germinated.

It appeared desirable to determine the percentage of seeds which contained embryos. This was done with the aid of a dissecting microscope by cutting the seeds near the basal end and forcing the embryo out by pressing the exterior of the seed with forceps. The iodine and Sudan III tests were found useful in detecting the presence or absence of embryos. The ungerminated seeds from germination tests of 2 months' duration were examined in this manner. A summary of the results of some of these tests is presented in table 6.

TABLE 6.—*Percentages of Cyperus rotundus seeds producing seedlings and containing embryos at end of germination tests, after specified periods of storage*

Year harvested	Lots in tests	Seeds tested and examined	Storage	Seedlings in germination tests	Seeds containing embryos
	Number	*Number*	*Months*	*Percent*	*Percent*
1941	4	600	15–16	6.0	50
1941	3	1,200	26–28	3.5	9
1942	2	800	13–14	3.5	30
1942	1	100	[1] 3	.....	85

[1] Stored dry at room temperatures. Seeds not tested for germination.

The rate of occurrence of seeds of the 1941 harvest containing embryos dropped from 50 percent to 9 percent between the fifteenth and twenty-eighth months. Although not shown in table 6, the percentage of seeds containing embryos varied with seed lots rather than storage conditions, with the following exception. Thirty-five percent of the seeds of lot 24, which had been stored moist at 10° C., contained embryos, while only 10 percent of those stored dry at room temperature previous to the germination tests contained embryos. Fungi had attacked the basal end of many of the older seeds and apparently destroyed the embryos.

In another similar test seeds which had been stored dry at room temperature were immersed in water for 24 hours and dissected. They were classified into four groups as follows: (a) embryo relatively large, (250μ to 300μ by 150μ to 200μ); (b) embryo average size

to small (150μ to 200μ by 100μ to 150μ); (c) seed with endosperm but no embryo; (d) embryo and endosperm decayed. The percentages of seeds placed in each classification are shown in table 7.

TABLE 7.—*Rate of occurrence and classification of Cyperus rotundus embryos based upon size*

Lot No.	Months stored	Seeds dissected	Embryos, large	Embryos, average to small size	Seeds with endosperm, no embryo	Seeds decayed
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
1	29	83	7	82	5	6
15	29	114	6	47	0	47
22	17	106	1	19	1	79
24	17	192	4	68	6	22
31	3	126	2	85	12	1

A total of 13,430 heavy seeds of *Cyperus rotundus* was used in all germination tests included in this investigation. These produced 554 or 4.12 percent normal seedlings, and 227 or 1.69 percent abnormal seedlings. The tests giving the highest results follow: (a) Lot 4—140 seeds, tested for 11 weeks after 24 months of moist storage at 10° to 20° C. alternating temperatures, gave 18 percent normal and 6 percent abnormal seedlings; (b) lot 1—150 seeds, tested for 11 weeks after 24 months of moist storage at 10°, gave 8 percent normal and 6 percent abnormal seedlings; (c) lot 22—100 seeds, tested for 13 weeks after 2.5 months of air-dry storage at room temperature and heated for 3 hours at 60°, gave 11 percent normal and 6 percent abnormal seedlings. In all other individual tests where 100 or more seeds per test were used the total of normal and abnormal seedlings was less than 10 percent. However, in several tests of 25 or 50 seeds per test the results varied from 10 to 30 percent.

VIABILITY AND DORMANCY IN SEEDS OF CYPERUS ESCULENTUS

Two tests were set up to determine if seeds of *Cyperus esculentus* were dormant soon after harvest. Four-week-old seeds of lots 12 and 17 were sowed in sterilized soil in a greenhouse and also between blotters at 20° to 30° C., alternating temperatures. No seedlings emerged from the soil tests within a period of 3 months. Of the seeds placed between blotters, lot 17 gave 4 percent germination within 2 months, 79 percent within 4 months, and 89 percent within 12 months. Lot 12 which consisted of low-quality seeds and germinated much more slowly than lot 17, gave a total of 50 percent germination within a year. Germination of dormant seeds was increased by moistening the substratum with a 0.2-percent solution of potassium nitrate. For example, 91 percent of the 6-month-old seeds of lot 33 germinated when the substratum was moistened with potassium nitrate solution and 37 percent when the substratum was moistened with water. Seeds of lot 32 treated similarly germinated 59 and 17 percent, respectively.

In order to study the effects of storage temperatures upon afterripening, samples of lots 12 and 17 were stored moist at 2° C., 10°,

2° to 20°, and 10° to 20° alternating temperatures. Results of germination tests made on samples withdrawn at intervals are shown in table 8.

TABLE 8.—*Effect of storage temperatures upon the germination of Cyperus esculentus seeds*

Lot No.	Period of storage	10° C. constant	10°–20° C. alternating	2° C. constant	2°–20° C. alternating
	Weeks	*Percent*	*Percent*	*Percent*	*Percent*
12	14	88	44	24	16
	18	78	38	36	26
17	14	82	84	88	80
	18	92	89	90	91
	22	84	----	89	----

The storage temperatures used were satisfactory for the afterripening of seeds of lot 17, but seeds of lot 12 responded much better after storage at 10° C. than at the other temperatures. Lots 25 and 26 were harvested in the same vicinities as lots 12 and 17, respectively, but a year later. Seeds of the two former lots were stored dry at room temperature and moist at 10°. Results from tests made at various intervals were comparable with results obtained from lots 12 and 17, which had been stored moist at 10°. In addition, it was determined that seeds would afterripen as soon when stored dry at room temperature as when stored moist at 10°. Seeds of the two heaviest fractions of lot 33 retained their viability for a year when stored dry at room temperature. The viability of the lighter fractions declined considerably during this period.

DISCUSSION

CYPERUS ROTUNDUS

The variety of conditions under which seeds of *Cyperus rotundus* were stored before testing and the variation in germination conditions would appear to provide a means of obtaining maximum seedling production. The methods followed in storing and testing seeds of this species were the same as those used for seeds of *C. esculentus* and nine other species of *Cyperus*,[7] each of which germinated as high as 90 to 100 percent. No appreciable differences were observed in results obtained from different germination conditions, but this may have been due to the low percentages of germination obtained rather than to the lack of effect caused by germination conditions. Germination from the heavy fraction of the various seed lots was between 2 and 18 percent.

Considering the number of seeds contained in the heaviest fraction and the highest germination value obtained in any test of the respective seed lots, to produce 1 seedling would require the seeds from an average of 2.36 inflorescences of lot 22, 7.38 inflorescenses of lot 23, and 5.24 inflorescences of lot 24, or a total average of 4.77 inflorescences of the three lots. Ranade and Burns (*13*) obtained 1.5 percent germination from 22,086 seeds taken from 100 inflorescences, which would amount to an average of 3.31 seedlings per inflorescence.

[7] Unpublished data.

Heating seeds for 3 hours at approximately 59° C. increased the germination slightly over seeds heated at 50° to 55°, 59° to 65°, and seeds not heated. These increases were not so striking as those reported by Ranade and Burns (*13*).

The necessity of afterripening of seeds and the length of time and conditions under which this process proceeds is forcibly illustrated in the tests in which seeds were used that had previously been tested and stored for several months air-dry at room temperatures. For example, 248 firm seeds which had been tested and subsequently stored for 20 months yielded 43 seedlings in a second germination test. This might suggest that the failure to obtain high percentages of germination was due to the testing of imcompletely afterripened seeds. No doubt, the results were affected slightly by this factor, but the total yield from 3,550 seeds in the original and second germination tests was 4.4 percent normal and 3.5 percent abnormal seedlings.[8]

There appeared to be no correlation between the total number of seeds containing embryos and percentage of germination, but there was an apparent correlation between the number of seeds containing large embryos and the percentage of germination. The heavy seeds appeared to contain embryos in various stages of development.

The field conditions under which seeds were harvested suggested that a delicate balance of factors was required for seed maturation. Among the factors which appear to contribute to seed production are the following: (a) Adequate moisture supply at the time of embryo maturation, (b) cultivation of land in early spring but not late spring or summer, (c) freedom from competition of perennial grasses and similar plants.

On the basis of results of this investigation it can be concluded that viable seeds of nutgrass are produced in the southern part of the United States but that reproduction of this species by seeds under natural conditions is probably of little importance. The proportion of viable seeds produced to the number of culms is extremely low. No attempt was made to determine the basis for the divergent conclusions arrived at from this study and those drawn by Holcomb [10]. The results of this investigation do not lend support to the claims of Dewey (*4*), Georgia (*6, pp. 68–69*), and the Virginia Extension Agronomy Department (*19, p. 49*), that seeds of nutgrass are commonly disseminated in grass and clover seeds and other media, and that the species is abundantly propagated in this manner. Likewise, it appears that the recommendations of Davis and Hawkins (*3*) and Muenscher (*11, pp. 174–176*) that seed production be prevented in a control program are not supported by these results.

Over a period of 5 years not a single agricultural or vegetable seed sample tested in the Federal seed laboratory at Beltsville, Md., or

[8] Since this paper was submitted for publication results of extensive experiments on the germination of seeds of *Cyperus rotundus* by F. W. Andrews[9] have been published. Andrews found 5-year-old seed to germinate 51.9 percent and 7-year-old seed to germinate 43.4 percent. Germination increased with age of seed up to 7 years, indicating dormancy and the necessity of afterripening.

[9] ANDREWS, F. W. A STUDY OF NUT GRASS (CYPERUS ROTUNDUS L.) IN THE COTTON SOIL OF THE GEZIRA. II. THE PERPETUATION OF THE PLANT BY MEANS OF SEED. Ann. Bot. [London] (n. s.) 10: [15]—30, illus. 1946.

[10] See footnote 5, p. 303.

the State seed laboratory at Montgomery, Ala., was found to contain nutgrass seed. Du Preez (*5*), Mac Mahon (*10*), and Smith and Fick (*18*) failed to obtain seedlings from nutgrass seed, whereas flowering plants were produced from seed in this investigation.

CYPERUS ESCULENTUS

Although there is some question among agronomists and botanists as to seed production by *Cyperus esculentus* in different parts of this country and from year to year, there can be little question but that it is potentially capable of reproducing freely by means of seeds. This species produces large quantities of viable seeds in the northeastern part of the United States, thus confirming the claims of Georgia (*6, pp. 70–71*) and Muenscher (*11, p. 174*) that this species reproduces by seeds.

SUMMARY

Seeds of nutgrass (*Cyperus rotundus*) harvested in Alabama and of yellow nutgrass (*C. esculentus*) were separated into four fractions based upon specific gravity and stored moist at 2° C., 10°, 2° to 20° alternating, 10° to 20° alternating, and 20° to 30° alternating temperatures and dry at room temperature.

Samples of seeds of *Cyperus rotundus* were withdrawn and tested for germination over a period of 3 years. Germination tests were made under a variety of conditions in an effort to determine optimum conditions for germination. The seeds were found to require an afterripening period before they would germinate. Maximum percentages of germination obtained from the heavy seed fractions ranged from 10 to 18 in tests consisting of 100 or more seeds. From a total of 13,430 heavy seeds tested there were obtained 4.12 percent normal and 1.69 percent abnormal seedlings. Actual seed counts on three lots showed that a minimum of 4.77 inflorescences would be required to yield a single seedling. There was no correlation between the number of seeds containing embryos and the percentage of germination, but there was an apparent correlation between the number of seeds with large embryos and percentage of germination. Even though some viable seeds of nutgrass are produced, reproduction by means of seeds is relatively unimportant in the southern part of the United States.

Germination of the heavy fractions of two seed collections of *Cyperus esculentus* from New York State was approximately 90 percent, that of 2 collections from Alabama approximately 50 percent, and that of a lot harvested in Maine 95 percent. Twenty-five inflorescences of the Maine lot yielded 50,260 seeds with an average germination of 75.6 percent, or the equivalent of 1,521 seedlings per inflorescence. The large numbers of viable seeds of yellow nutgrass indicate that it reproduces freely by seeds.

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CHANGES IN THE CHEMICAL COMPOSITION OF THE TUNG FRUIT AND ITS COMPONENT PARTS[1]

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INTRODUCTION

The chemical composition of fruit of tung (*Aleurites fordii* Hemsl.) at various stages of development has not been reported; but such data are available for pecans (*18*, *19*),[3] almonds (*11*), walnuts (*11*, *12*), and macadamia (*9*) as well as for various oil-producing seeds, such as cotton (*3*, *5*, *6*) and rape, flax, hemp, poppy, and sunflower (*7*).

A knowledge of the periods when various chemical changes occur in tung fruit would be helpful in formulating cultural and nutritional practices. Therefore, at biweekly intervals from June 28 to November 29, 1940, analyses were made for carbohydrates, for nitrogen and some of its fractions, and for oil. The relation of increase in dry weight, reducing sugars, protein nitrogen, sucrose, and starch to oil content was determined. Studies were also undertaken to determine (1) the effect of storing fruits at different harvest dates and (2) the relation of the dry weight and oil content of fruits that dropped on different dates. The results are reported in this paper.

MATERIALS AND METHODS

SAMPLING PROCEDURES

Tung fruits for chemical analysis were picked at 2-week intervals from June 28 to November 29, 1940, from a single tree in order to eliminate variations that might arise from differences between trees. This limited each sample to 25 fruits and necessarily involved moderate sampling errors (*15*).[4] The tree from which the fruits were obtained was located near Gainsville, Fla.

In the case of oil in the whole fruit the standard errors of the means should not exceed 0.71 percent; the standard error of the difference

[1] Received for publication April 14, 1945.
[2] The authors acknowledge many helpful suggestions by George F. Potter in the preparation of this paper.
[3] Italic numbers in parentheses refer to Literature Cited, p. 333.
[4] LAGASSE, F. S., et al. METHODS OF DETERMINING TUNG OIL AND ITS QUANTITATIVE VARIATION. [Unpublished manuscript.]

between two such means will then be approximately 1.00 percent and significant differences of 2.00 percent between means can be distinguished. The fruits were picked at random from all parts of the tree between 8 and 10 a. m. The condition of the fruits is described and their average dry weights on each sampling date are given in table 1.

TABLE 1.—*Weight and condition of tung fruits on each sampling date, 1940*

Sampling date	Time after full bloom	Average dry weight per fruit	Condition of fruits
	Weeks	*Grams*	
June 28	11	15.4	The shell showed definite hardening. No true kernel (endosperm and embryo) was visible. Nucellus, which filled the shell at this stage, appeared soft and watery.
July 12	13	16.9	Do.
26	15	20.4	The shell was hard enough to be separated from the contents, which were still largely nucellus. The growing endosperm and embryo were visible in the center of the nucellus.
Aug. 9	17	22.6	The endosperm and embryo practically filled the shell, which was well-formed, hard, and readily removed. The kernel appeared dry and fully developed.
23	19	26.0	Do.
Sept. 6	21	26.2	Do.
20	23	29.5	Do.
Oct. 4	25	30.6	Fruits almost mature.
18	27	29.3	The fruits for this sample were picked up from the ground. A few were still on the tree.
Nov. 1	29	29.3	All fruits were picked up from the ground. Hulls were brown and hard.
15	31	28.8	Do.
29	33	28.7	Do.

At each sampling date the whole lot of fruits was weighed in order to determine the average weight per fruit. Since the shell and kernels were not developed sufficiently on June 28 and July 12, the fruits were separated into hull and nut[5] only on these dates. Beginning on July 26, however, the fruits were separated into their component parts (hull, shell, and kernel). By November 1 the outer integuments had changed into reddish-brown dust, which was sampled separately from the shell. Immediately after separation the hulls and shells were cut into small pieces with pruning shears. The kernel samples were prepared by flaking them on an inverted jack plane (*4*) in preference to cutting them into small pieces with pruning shears or by grinding. Triplicate 50-gm. samples of each part were weighed out in 500-ml. Erlenmeyer flasks, each containing 0.50 gm. of calcium carbonate, and preserved by refluxing in approximately 80-percent ethanol in a boiling-water bath for 30 minutes (*1*). The flasks were stoppered while still hot and stored in the dark until the contents were analyzed.

For a comparison of the oil content at picking and after storage, 50 fruits were obtained on various dates from a second tree; those for comparing the oil content of fruits dropping on different dates were obtained from a third. Only dry matter and oil content were determined on these.

ANALYTICAL METHODS

The methods of the Association of Official Agricultural Chemists (*1*) were employed whenever possible for these analyses.

[5] Nut includes shell plus kernel.

Dry matter.—To determine dry matter in the freshly collected samples, duplicate 5-gm. samples were weighed in aluminum dishes immediately after sampling, placed over phosphorus pentoxide in a vacuum oven at 70° C. and 4-mm. pressure, and dried to constant weight. In determining dry matter in the preserved tissue, the total dry weight of the sample was found by adding the weight of the dried residue after filtration of the alcohol (corrected for calcium carbonate added) to the weight of the solids from the filtrate. The dry weight of the alcohol-soluble fraction was determined by evaporating a 25-ml. aliquot of the filtrate and drying it to a constant weight. The percentage of dry matter was calculated from the dry and fresh weights of the sample.

Nitrogen.—The Kjeldahl-Gunning method as outlined by the Association of Official Agricultural Chemists (*1*) was used. For the kernels 80 ml. of concentrated sulfuric acid was taken instead of the recommended 40 ml., because of the high oil content of the material. Total nitrogen was determined from a 50-ml. aliquot of the original alcoholic extract plus one-fifth of the original insoluble residue. The alcohol was completely evaporated before the addition of the dried residue. A 50-ml. aliquot of the composite alcoholic solution was used for alcohol-soluble nitrogen, representing soluble nitrogen compounds. The difference between total and soluble nitrogen gave the alcohol-insoluble fraction, which was designated as protein nitrogen.

Sugars.—The alcohol-preserved samples, representing approximately 50 gm. of fresh material, were filtered on dry, tared filters into 250-ml. volumetric flasks and made to volume with 80-percent ethanol. The residues were dried in a vacuum oven at 70° C. and 4-mm. pressure over phosphorus pentoxide until constant weight was obtained; then they were ground in an intermediate Wiley mill to pass an 80-mesh sieve. One-half of each residue was transferred to a Soxhlet extraction unit and extracted for 16 hours with 80-percent ethanol. The kernel samples were defatted with petroleum ether before they were extracted with 80-percent ethanol. The resulting extract was transferred quantitatively to a 250-ml. volumetric flask containing 125 ml. of the original alcoholic extract. After the contents of the flask had been made to volume with 80-percent ethanol, a 125-ml. aliquot of the solution was evaporated almost to dryness. The residue was taken up with water, treated with sufficient neutral lead acetate to clarify the solution, made to a volume of 200 ml. with water, and centrifuged to remove the lead precipitate. The supernatant liquid was deleaded with anhydrous sodium oxalate and filtered through a dry filter into a dry flask. Reducing sugars were determined in a 50-ml. aliquot by precipitating the cuprous oxide according to the Munson-Walker procedure (*1, p. 500*). The reduced copper (cuprous oxide) was estimated by the volumetric ceric sulfate method (*2*). For the determination of nonreducing sugar, inversion was accomplished by means of hydrochloric acid at room temperature (*1, pp. 495, 498*). The Munson-Walker table (*1, pp. 671–677*) was used to calculate reducing sugars as dextrose and nonreducing sugar as sucrose.

Starch.—The alcohol-insoluble residues (*1, 16*) that had been extracted in a Soxhlet unit were transferred into a 300-ml. wide-mouthed Erlenmeyer flask. The volume was made to 90 ml. with water, and the flask was heated in a boiling-water bath for 1 hour. After the

flask had been cooled to room temperature 10 ml. (equivalent to 0.1 gm.) of a solution of taka diastase was added. The mixture was protected by toluene in a stoppered flask and incubated at 38° C. for 24 hours. The flask was then heated in a boiling-water bath for 15 minutes to destroy the enzyme. When cool the solution was treated with 160 ml. of 95-percent ethanol and filtered into a 500-ml. volumetric flask. The filtrate and washings were made to volume with 60-percent ethanol, and a 50-ml. aliquot was evaporated almost to dryness; then it was taken up in 75 ml. of water and hydrolyzed with 5 ml. of concentrated hydrochloric acid in a water bath at 80° for 3½ hours. After being cooled and neutralized, the solution was diluted with water to 100 ml. in a volumetric flask and reducing sugars were determined in a 50-ml. aliquot. The weight of the starch in the sample was calculated by multiplying the weight of the reducing sugars by 0.90.

Polysaccharides.—The insoluble residue (*16*) from the starch determination was transferred to a 300-ml. Erlenmeyer flask, and water was added to make a total of 100 ml. To the contents was added 10 ml. of concentrated hydrochloric acid, and the solution was heated for 2½ hours in a boiling-water bath. The solution was transferred to a 500-ml. volumetric flask, made to volume, and filtered. A 100-ml. aliquot was neutralized with sodium carbonate and then made to volume in a 200-ml. volumetric flask. Reducing substances were determined in a 50-ml. aliquot. The weight of polysaccharides in the sample was calculated by multiplying the total weight of reducing substances (as dextrose) in the insoluble residue from the starch determination by 0.90.

Oil.—Duplicate 5-gm. samples of flaked tung kernels were weighed in paper thimbles and extracted with acetone in a Goldfisch extraction unit for 12 hours. The solvent and the oil were collected in previously tared flasks, the solvent was evaporated off, and the oil was dried in a vacuum oven over phosphorus pentoxide at 70° C. and 4-mm. pressure for 3½ hours, cooled in a desiccator, and weighed. The percentage of oil was then calculated from the weight of oil and dry weight of the sample.

Since little is known concerning the changes occurring in the immature tung kernels during heating and drying, it appeared desirable to avoid heating them. Petroleum ether is immiscible with water and hence will not completely extract the oil from the immature flaked tung kernels. Acetone was therefore employed as a solvent since it removes all of the oil. The use of acetone gives higher values in the determination of the oil content of the immature kernel, because of the presence of small quantities of other substances soluble in moist acetone. After August 9, the error from this source was approximately of the same order of magnitude as the sampling error. However, in mature fruits the difference between acetone and petroleum ether is not significant.

RESULTS OF BIWEEKLY ANALYSES

DRY MATTER

In general, the percentage of dry matter increased in the whole fruit, hull, outer integument, shell, and kernel throughout the entire sam-

pling period (table 2). However, the percentage of dry matter in the hull did not change much until after October 18; then it increased rapidly to more than 70 percent as the fruit dried on the ground.

The dry matter (table 2) in the whole fruit increased to October 4 and then decreased slightly to the end of the study. The dry matter in the hull fluctuated from 11.9 gm. on July 12 to 14.9 gm. on October 4 and then decreased until November 1, after which little or no change was noted. No definite trend was observed in the outer integument. In the shell (mostly nucellus on June 28 and July 12) there was an increase from June 28 to August 23, after which the changes were not significant. From July 26 to September 20 the kernels increased rapidly in dry weight and then more gradually until they weighed more than 9 gm. per fruit between November 1 and 29.

TOTAL NITROGEN

The percentage of total nitrogen in the whole fruit decreased from 1.21 on June 28 to 0.89 on September 6 and in the hull from 1.06 on June 28 to 0.37 on October 4 (table 2). In the case of whole fruit there was a moderate increase from September 6 to November 1, after which there were no significant changes. In the hull no consistent changes were observed after October 4. The percentage of total nitrogen in the outer integument remained almost constant. In the shell the percentage of total nitrogen decreased from June 28 to August 23 and thereafter no trend of importance was observed. In the kernel the percentage of total nitrogen decreased from 4.46 on July 26 to 2.48 on September 6 and thereafter the changes were not significant.

The data in table 2 show a general increase in total nitrogen content in the whole fruit from June 28 to November 1. In the hull a consistent decline until October 4 was noted; thereafter nitrogen remained almost constant. No changes were observed in the outer integument. In general, the total nitrogen in the shell decreased until September 6, after which there was little or no change. The general accumulation of total nitrogen in the kernel from July 26 to November 1 shows that nitrogen was being stored as a reserve food material. The considerable differences between total and soluble nitrogen during the latter part of the season indicate that this reserve food material was largely protein nitrogen. The decrease noted in total nitrogen in the hull would indicate that it was a source of nitrogen supply for the production of protein nitrogen in the kernel.

ALCOHOL-SOLUBLE NITROGEN

The percentage of soluble nitrogen in the whole fruit decreased from August 9 to November 15 (table 2). In the hull the decrease in percentage of this constituent occurred from July 12 to November 29, whereas in the shell (mostly nucellus on the first two sampling periods) it occurred from July 12 to September 20. Sufficient data were not available for the outer integument to permit drawing any conclusions. In the kernel the percentage decreased rapidly from July 26 to August 23 and then very slowly until the fruit had matured (November 1).

TABLE. 2.—*Composition of whole and component parts of tung fruits from June 28 to November 29, 1940*

[Quantity per fruit; moisture-free basis]

Part of fruit and date of sampling	Dry matter		Total nitrogen		Alcohol-soluble nitrogen		Reducing sugars		Nonreducing sugar		Starch		Polysaccharides [1]		Oil	
	Percent	*Grams* [2]	*Percent*	*Milligrams* [3]	*Percent*	*Milligrams* [3]	*Percent*	*Milligrams* [3]	*Percent*	*Milligrams* [3]	*Percent*	*Milligrams* [3]	*Percent*	*Milligrams* [3]	*Percent*	*Grams* [2]
Whole fruit:																
June 28	19.27	15.4	1.21	186	0.21	32	2.68	413	5.55	855	1.85	285	10.15	1,563	------	------
July 12	19.56	16.9	1.08	183	.27	46	2.36	399	5.71	965	1.43	242	9.93	1,678	------	------
July 26	22.90	20.4	1.05	214	.29	59	2.46	502	5.29	1,079	.86	175	9.75	1,989	(3)	(3)
Aug. 9	25.68	22.6	.98	222	.29	66	1.62	366	2.61	590	.74	167	8.85	2,000	3.9	0.88
Aug. 23	29.35	26.0	.98	255	.14	36	1.26	328	2.57	668	.60	156	7.01	1,823	11.5	2.99
Sept. 6	30.35	26.1	.89	232	.12	31	1.98	517	1.95	509	.58	151	6.43	1,678	15.2	3.97
Sept. 20	34.30	29.5	.96	283	.11	33	1.36	401	1.53	451	.62	183	6.65	1,962	18.6	5.49
Oct. 4	37.14	30.6	.97	297	.10	31	1.39	425	1.85	566	.70	214	7.19	2,200	18.9	5.78
Oct. 18	37.23	29.3	1.02	299	.09	26	1.12	328	1.53	448	.65	191	7.11	2,083	19.6	5.74
Nov. 1	74.37	29.3	1.23	363	.07	21	.19	56	1.04	305	.56	164	6.82	1,998	23.2	6.80
Nov. 15	84.96	28.8	1.06	305	.06	17	.13	37	.99	285	.56	161	6.79	1,956	23.3	6.71
Nov. 29	84.66	28.7	1.13	324	.06	17	------	------	1.39	399	.61	175	6.91	1,983	23.7	6.80
Hull:																
June 28	25.53	12.5	1.06	133	.17	21	2.82	353	4.56	570	1.50	188	11.60	1,450	------	------
July 12	21.84	11.9	.88	105	.17	20	2.62	312	4.62	550	1.56	186	9.31	1,108	------	------
July 26	23.76	13.6	.77	105	.14	19	3.10	422	3.87	526	1.19	162	9.79	1,331	------	------
Aug. 9	22.10	14.5	.66	96	.12	17	2.43	352	2.90	421	1.15	167	8.86	1,285	------	------
Aug. 23	21.84	13.3	.50	67	.10	13	2.19	291	3.26	433	1.18	157	7.99	1,063	------	------
Sept. 6	21.05	13.2	.46	61	.09	12	3.63	479	2.80	370	1.06	140	6.48	855	------	------
Sept. 20	23.14	14.7	.42	62	.08	12	2.74	403	1.78	262	1.19	175	7.48	1,110	------	------
Oct. 4	25.67	14.9	.37	55	.07	10	2.85	425	1.72	256	1.34	200	8.95	1,334	------	------
Oct. 18	25.80	14.3	.38	54	.05	7	2.29	328	.47	67	1.20	172	8.56	1,224	------	------
Nov. 1	69.73	11.8	.43	51	.03	4	.45	53	(3)	(3)	1.13	133	9.18	1,083	------	------
Nov. 15	77.92	12.4	.40	50	.02	2	.30	37	(3)	(3)	1.08	134	9.16	1,136	------	------
Nov. 29	76.81	11.9	.47	56	.01	1	(3)	(3)	(3)	(3)	1.20	143	9.45	1,125	------	------
Outer integument:																
Nov. 1	48.17	.6	1.09	7	.04	.2	.42	3	.47	3	1.12	7	3.63	22	------	------
Nov. 15	80.60	.7	1.05	7	.05	.4	(3)	(3)	(3)	(3)	.78	6	3.95	28	------	------
Nov. 29	83.24	.5	1.11	6	------	------	(3)	(3)	(3)	(3)	1.23	6	4.24	21	------	------
Shell:																
June 28 [4]	9.38	2.9	1.85	54	.40	12	2.10	61	9.86	286	3.32	96	------	------	------	------
July 12 [4]	15.67	5.0	1.56	78	.52	26	1.74	87	8.28	414	1.12	56	11.42	571	------	------
July 26	31.70	5.6	1.00	56	.28	16	.73	41	7.09	397	(3)	(3)	11.20	627	------	------
Aug. 9	63.18	5.9	.52	31	.10	6	.22	13	.63	37	(3)	(3)	10.97	647	------	------
Aug. 23	60.88	7.7	.41	32	.04	3	.46	35	.95	73	(3)	(3)	8.18	630	------	------
Sept. 6	69.88	7.0	.36	25	.03	2	.54	38	(3)	(3)	.15	11	9.54	668	------	------
Sept. 20	75.81	6.8	.39	27	.02	1	(3)	(3)	(3)	(3)	.11	8	9.83	668	------	------
Oct. 4	74.85	6.8	.35	24	.02	1	(3)	(3)	(3)	(3)	.20	14	9.55	649	------	------
Oct. 18	75.47	6.5	.39	25	.02	1	(3)	(3)	(3)	(3)	.31	20	10.02	651	------	------

Nov. 1	87.33	7.4	.40	30	.02	1	(3)	(3)	(3)	(3)	.33	24	9.39	695		
Nov. 15	88.35	6.4	.37	24	.02	1	(3)	(3)	(3)	(3)	.34	22	8.90	570		
Nov. 29	88.32	6.9	.44	30	.02	1	(3)	(3)	(3)	(3)	.38	26	9.24	638		
Kernel:																
July 26	8.52	1.2	4.46	54	2.05	25	3.29	40	12.94	155	1.10	13	2.62	31	(3)	(3)
Aug. 9	16.88	2.2	3.93	87	1.95	43	(3)	(3)	6.30	139	(3)	(3)	3.36	74	40.5	.89
Aug. 23	33.50	5.0	3.03	152	.39	20	(3)	(3)	3.19	160	(3)	(3)	2.55	128	59.4	2.97
Sept. 6	44.01	5.9	2.48	146	.29	17	(3)	(3)	2.35	139	(3)	(3)	2.68	158	67.4	3.98
Sept. 20	59.81	8.0	2.44	195	.24	19	(3)	(3)	2.37	190	(3)	(3)	2.43	194	68.5	5.48
Oct. 4	58.40	8.9	2.43	216	.21	19	(3)	(3)	3.49	311	(3)	(3)	2.44	217	65.2	5.80
Oct. 18	58.35	8.5	2.59	220	.22	19	(3)	(3)	4.49	382	(3)	(3)	2.43	207	67.7	5.76
Nov. 1	82.14	9.5	2.88	274	.15	14	(3)	(3)	3.18	302	(3)	(3)	2.06	196	71.7	6.81
Nov. 15	94.08	9.3	2.42	225	.14	13			3.08	286	(3)	(3)	2.39	222	72.1	6.71
Nov. 29	93.76	9.4	2.46	231	.17	16			4.23	398	(3)	(3)	2.12	199	72.2	6.79

[1] Acid-hydrolyzable residue from starch determinations.
[2] Slight differences in the total of component parts from that in the whole fruit are due to rounding off figures in the calculations.
[3] Trace.
[4] Shell was mostly nucellus.

The weight of soluble nitrogen in the whole fruit and hull decreased from August 9 and June 28 to November 15 and 29, respectively (table 2). In the shell there was a rather sharp decrease in soluble nitrogen from July 12 to September 20, after which the content remained constant. The amount of soluble nitrogen in the kernel decreased gradually from August 9 to November 1. The changes after this date were not significant.

The fact that total nitrogen increased and soluble nitrogen decreased in the kernels indicates that beginning about the middle of August soluble nitrogenous compounds are converted into protein nitrogen. By inspection of table 2 it is seen that the amount of soluble nitrogen in the kernels represents in most cases a small portion of the total nitrogen. The difference represents largely protein nitrogen. The decreases in total and soluble nitrogen content noted in the hull and the concomitant increases in these constituents in the kernel indicate that proteins are hydrolyzed to soluble products and translocated to the kernel for the production of protein nitrogen.

REDUCING SUGARS

Reducing sugars were found to vary in the different parts of the tung fruit. In general, the percentage of reducing sugars in the whole fruit declined throughout the entire period of study. The percentage of reducing sugars in the hull showed no definite trend until October 4, after which a sharp decrease took place (table 2). In the outer integument the reducing sugars decreased to a trace by November 15. The percentage of reducing sugars in the shell (mostly nucellus on June 28 and July 12) decreased sharply between June 28 and August 9; subsequently, they were at the approximate 0.50 level until September 6, after which only a trace remained. On July 26 the kernels contained 3.29 percent reducing sugars, but by August 9 they contained only a trace.

The weight of reducing sugars in the whole fruit fluctuated during the early part of the season, but it tended to decrease gradually from September 6 to November 15, when only 37 mg. remained. From June 28 to October 18, the quantity of reducing sugars in the hull varied widely, and then it decreased to 53 mg. by November 1, when the oil content had reached its maximum (table 2). The reducing sugars in the shell were highest on July 12 and decreased until September 20, when only a trace remained. The kernels contained 40 mg. of reducing sugars on July 26 and only a trace from August 9 to November 1. The observed decreases in reducing sugars in the hull and kernel, together with the concomitant gain in oil, protein nitrogen, sucrose, and polysaccharides in the kernels, indicate that the simple sugars were utilized for the synthesis of these storage substances.

NONREDUCING SUGAR

On a percentage basis the nonreducing sugar reached a maximum in the whole fruit and hull by July 12 and in the shell (mostly nucellus) by June 28, after which a gradual decrease was observed (table 2). In the shell and hull only a trace was noted by September 6 and November 1, respectively. In the outer integument there was a decrease in nonreducing sugar from November 1 to November 15. It was noted that

the percentage of nonreducing sugar in the kernel, identified as sucrose (*17*), was at a maximum on July 26 and then decreased sharply to a minimum on September 6. A gradual increase then occurred bringing the kernel up to 4.23 percent sucrose on November 29.

Nonreducing sugar per fruit increased rapidly in the whole fruit and attained a maximum on July 26, when it decreased at first sharply and then slowly until November 15 (table 2). The nonreducing sugar in the hull was at a maximum on June 28 and decreased rather steadily to a trace by November 1. In the outer integument a decrease in nonreducing sugar was observed between November 1 and 15. In the shell (mostly nucellus) there was an increase between June 28 and July 12, after which there was a decrease to a trace by September 6. In the kernel the nonreducing sugar tended to increase from September 6 until after harvest.

The reduction in weight of nonreducing sugar in the hull and the general increase in oil, sucrose, polysaccharides, and protein nitrogen in the kernel between July 26 and November 1 indicate that nonreducing sugar was probably translocated to the kernel for the production of these substances.

STARCH

The percentage of starch in the whole fruit decreased from June 28 to August 23, after which it remained rather constant (table 2). In the hull the changes were not significant. Little change was observed in the percentage of starch in the outer integument. In the shell (mostly nucellus on June 28 and July 12) the percentage of starch decreased from 3.32 to a trace by July 26 and from August 23 to November 29 it increased in general. The kernel contained 1.10 percent starch on July 26; by August 9 starch had decreased to a trace.

The dry weight of starch (table 2) in the whole fruit showed a decline from June 28 to September 6. There was a second maximum on October 4, after which the changes were not significant. Similar trends were noted in the hull. No trend in starch content was observed in the outer integument. In the shell there was only a trace of starch from July 26 to August 23, after which there was in general a slight increase to November 29. In the kernel the weight of starch decreased from 13 mg. on July 26 to a trace by August 9, after which no further changes occurred. The reduction in starch in the kernel indicates that it is converted into oil and other constituents. Starch in the hull appears to have contributed to production of storage substances in the kernel much less substantially than did reducing and nonreducing sugars.

POLYSACCHARIDES [6]

The percentage of polysaccharides in the whole fruit and hull decreased from June 28 to September 6, after which there was a slight increase in the hull to November 29 but little further change in the whole fruit (table 2). A slight increase in the percentage of polysaccharides in the outer integument was observed from November 1 to 29. In the shell the percentage decreased from July 12 to August 23 and thereafter only minor fluctuations were noted.

[6] Acid-hydrolyzable residue from starch determinations.

The data in table 2 show that for the whole fruit the trend in the quantity of this constituent was slightly upward until August 9 and downward until September 6; the quantity attained a second maximum on October 4, after which it remained rather constant. In the hull there was a minimum on September 6, but there was little difference in the quantities found on the other sampling dates. In the outer integument no change was observed. In the shell (mostly nucellus on July 12) there was little change in the amounts of polysaccharides during the period of sampling. The polysaccharide content of the kernel increased rapidly up to October 4 as the kernel gained weight; then it remained approximately constant.

These results indicate that the polysaccharides are primarily constituents of cell walls and play only a minor part if any in the synthesis of oil in the kernel.

RELATION OF OIL ACCUMULATION TO OTHER CONSTITUENTS OF THE KERNEL

The data on oil content and the other constituents of tung kernels during the period of study are given in table 2. The dry weight of the kernel increased rapidly from July 26 to September 20 and then more slowly until November 1, suggesting a typical sigmoid growth curve. The oil in the kernel (per fruit basis) accumulated more slowly than the dry weight of the kernel at first, then increased sharply until September 20, after which it increased more slowly until November 1 (maturity). On September 6 dry matter and oil in the kernel had attained 62.1 percent and 58.4 percent of their respective final dry weights (November 1). In general, the protein nitrogen and sucrose accumulated concurrently with the oil in the kernel. These increases seem small, because of the limited quantities of each present as compared with the dry weight and oil content. However, the quantities of protein nitrogen and sucrose present in the kernel on September 6 were 49.6 percent and 46.0 percent, respectively, of the total amounts of these constituents on November 1. The diminution of reducing sugars and starch in the kernels indicates that these carbohydrates were utilized in the production of oil, sucrose, polysaccharides, protein nitrogen, and other substances. Sugars and other substances were probably translocated in sufficient amounts from other parts of the tree into the fruit for its growth (dry weight) and oil formation, each of which attained the maximum by November.

EFFECT OF HARVEST DATE AND STORAGE ON OIL CONTENT OF TUNG FRUIT

To study the changes in oil content in the fruits picked at different harvest dates and after a period of storage, 50 fruits were picked at biweekly intervals from a single tree. The oil content of 25 of the fruits from each sample was determined immediately in the manner previously described, and the remaining 25 fruits were stored in the laboratory until December 6, 1940, and then analyzed for oil. The resulting data given in table 3 show that only approximately one-third of the maximum oil was present in the fruits harvested on August 23, but that approximately the maximum oil content had been attained

by October 4. The fruits of this tree matured earlier than those of the tree used in the previous study. It was also noted that there was no significant gain in oil upon storage of the immature fruits. As previously pointed out, with fruits on the tree, carbohydrates translocated from the hull and shell contribute to the oil and other storage products in the kernel. However, only a small proportion of the total storage substances of the kernel can originate from this source; substances necessary for synthesis of oil and other reserve foods must in large measure be translocated into the fruits from the tree.

TABLE 3.—*Oil content of tung fruits at different harvest dates and after storage until December 6, 1940*

[Moisture-free basis]

Date of harvest	Oil per fruit				Date of harvest	Oil per fruit			
	Fruits analyzed at picking		Fruits stored until December 6			Fruits analyzed at picking		Fruits stored until December 6	
	Percent	*Grams*	*Percent*	*Grams*		*Percent*	*Grams*	*Percent*	*Grams*
Aug. 23	10.5	2.4	11.5	2.0	Oct. 18	22.6	7.3	26.3	7.9
Sept. 6	17.6	4.7	18.0	3.5	Nov. 1	23.2	7.8	25.4	7.6
20	21.6	6.7	24.3	6.5	15	25.4	7.5	26.7	8.0
Oct. 4	23.9	7.5	25.3	7.0	29	25.3	7.5	26.2	7.7

RELATION OF DRY WEIGHT TO OIL CONTENT OF FRUITS DROPPING ON DIFFERENT DATES

In order to compare the oil content of fruits that dropped from the tree early in the season with that of fruits that fell on later dates, 5 to 25 fruits, as available, were picked up beneath a third tree at weekly intervals beginning September 20, when the first fruits fell, and ending November 15, when practically all fruits were on the ground. The data in table 4 show that the kernels of the earliest fruits to drop, of which there were very few (5 on September 20 and 6 on September 27), had lower dry weights and contained less total oil than fruits falling later. The maximum oil content was attained in the fruits that dropped from October 4 to 18, after which there was little change. The data from this single tree cannot be extended to trees that mature their fruit earlier or later.

DISCUSSION

According to McCann (*13*), the growth of the tung fruit from the time of set to maturity can be divided into two major periods: (1) A period of growth in size of the structural elements of hull and seed coat and (2) structural and physiological filling of the seed by the growth of the endosperm and embryo, which is initiated about the time the fruit attains its maximum size. McCann reported that, although the seeds are filled with endosperm 6 to 8 weeks after the attainment of maximum fruit size, little oil is present in the cells. A similar initial period of structural formation from blossoming to late August or early September has been reported for the pecan by Thor and Smith (*18*).

TABLE 4.—*Oil content of tung fruits falling at weekly intervals from September 20 to November 15, 1940*

[Moisture-free basis]

Period fruits dropped[1]	Average dry weight of kernels per fruit	Oil per fruit		Period fruits dropped[1]	Average dry weight of kernels per fruit	Oil per fruit	
	Grams	*Percent*	*Grams*		*Grams*	*Percent*	*Grams*
Sept. 20	5.1	20.0	3.1	Oct. 25–Nov. 1	8.7	31.3	6.5
Sept. 20–27	6.2	23.4	4.1	Nov. 1–8	8.1	30.3	6.0
Oct. 4–11	8.9	31.0	6.6	Nov. 8–15	8.5	29.6	6.2
Oct. 11–18	9.6	32.0	7.1	Nov. 15	8.7	29.3	6.4
Oct. 18–25	8.6	31.3	6.4				

[1] No fruit dropped between Sept. 27 and Oct. 4.

In this study the same structural and physiological changes have been observed as were reported by McCann. The data presented in this paper confirm and extend his findings. In the present investigation only a small amount of oil was found in the endosperm in early August. This also was observed microscopically by McCann. However, a rapid increase on a weight basis representing 67.4 percent of the final dry weight of oil as of November 1 took place in the kernels during the 6-week period from August 9 to September 20. Concurrently, the protein nitrogen (difference between total and soluble nitrogen) and the polysaccharides in the kernel on a milligram basis increased 50.8 and 61.2 percent, respectively, of their final dry weight. The maximum oil content was attained by November 1 (table 2). It has been shown by Thor and Smith (*18*) that almost identical results were noted in these constituents during the filling period of the pecan kernel.

During the period of oil synthesis the soluble nitrogenous products represented by the alcohol-soluble fraction were largely converted into protein nitrogen. The greatest quantity of protein nitrogen on an actual dry-weight basis was found in the kernel. In the hull and shell a gradual decrease of these substances was noted from June 28 to October 4, indicating that these protein materials were hydrolyzed and the soluble products were translocated to the kernels for storage as reserve proteins. Since the gain in protein nitrogen in the kernels exceeds the loss in other parts of the fruit, soluble nitrogen must be translocated into the fruit from the tree. That protein nitrogen accumulates in other oily seeds has been shown by numerous investigators (*6*, *7*, *14*, *18*). It was noted in table 2 that the whole fruit at maturity (November 1) contained about 1.23 percent nitrogen, or approximately 360 mg. of nitrogen per fruit. In an orchard yielding 1,000 pounds of dried fruit per acre, 12.3 pounds of nitrogen, or the equivalent of 76.9 pounds of commercial nitrate of soda (16 percent N) per acre, would be removed from the orchard in the fruit at harvesttime. Therefore, it is of considerable importance to keep the tung tree well fertilized with nitrogen in order to maintain proper growth and to supply an adequate amount for the development of the fruit.

In the carbohydrate fractions of the kernel the reducing sugars were reduced to a trace at the time of oil synthesis; this reduction is a

characteristic phenomenon that has been frequently observed in other oily seeds by Ivanow (*7*), Müntz (*14*), and Leclerc du Sablon (*11, 12*). Starch acted similarly to reducing sugars in that it also was reduced to a trace at the time of oil synthesis. This is in agreement with the results of Müntz (*14*), who believed that starch was utilized in the production of oil in the kernels of rape and that glucose was the intermediate substance. Johnston and Sell (*8*), in their study of the changes in the chemical composition of germinating tung kernels, observed that oil yielded reducing sugars and starch. This is the reverse of oil synthesis, and these relations have been observed by Leclerc du Sablon (*10, 11*). The nonreducing sugar in the kernels is entirely sucrose (*17*). It increased gradually along with the oil during the period of filling. In a similar period in pecan development (*18*), it was observed that the total sugars, primarily nonreducing sugar, increased as the kernels were filling with oil. Leclerc du Sablon (*11, 12*) concluded from his study of oily seeds that the sucrose should be considered as a reserve material and reducing sugars as precursors of the oil. This is in agreement with the results obtained on tung and pecans. The diminution of reducing and nonreducing sugars in the hull after September 6 would suggest that as maturity approached these sugars were translocated to the kernels and synthesized into oil and other substances. Since the increase of these substances in the kernels exceeds the loss from other parts of the fruit, soluble sugars must be translocated into the fruits from other parts of the tree. During the latter part of the season the starch in the shell increased, but no appreciable reduction of this constituent was observed in the hull. The polysaccharides accumulating in the immature tung kernels were primarily constituents of the cell wall and other structural tissues. The polysaccharides, which comprised a considerable portion of the hull and shell, changed only slightly and therefore must be considered structural substances. A few workers (*7, 18*) have concluded that the polysaccharides in other oily seeds do not serve as carbohydrate reserves for the synthesis of oil and sucrose.

The analyses of immature tung fruits picked from the trees the latter part of August and early in September (table 3) and of those fruits that dropped prematurely (table 4) showed that neither contained the normal complement of oil. Analyses of the stored immature fruits (table 3) indicated that no significant gain in oil occurred. From these results it was concluded that nearly all the substances from which oil is formed are brought into the fruit from other parts of the tree at the time of oil synthesis. If a normal oil content is to be attained, the fruit must remain on the tree during the approximate 6-week period after structural development of the endosperm and embryo is complete. In this connection it is interesting to note that the Government of China [7] enacted a law prohibiting growers from knocking immature fruits from the tree because of the low oil content of such fruit. In the case of the pecan (*18*) it has also been observed that early harvesting is inadvisable because of the lower quality of the kernel.

[7] LIU, H. REPORT ON THE TUNG-OIL TREE CULTURE IN WEST CENTRAL CHINA. [China] Gov. Test. Bur., Chem. Res. Lab. 12 pp. Hankow, China. 1935.

It is important that sufficient moisture be available at all times if tung trees are to grow well and be profitably productive. The foliage must remain turgid in order that the photosynthetic activity of the leaves may elaborate the greatest amount of carbohydrates for the filling of the nuts and the development of oil. Sufficient carbohydrates must be formed prior to and throughout the period of oil formation to insure maximum synthesis of oil. The average rainfall in September is usually less than that of June, July, and August in northern Florida and the other Gulf States. During this dry period a considerable portion of the oil is synthesized in the tung kernels, and everything possible should be done to conserve all available soil moisture. For this reason it would seem advisable to turn cover crops under before September since such crops, as well as weeds, consume large quantities of water. In this way competition for moisture would be greatly reduced during this period of stress.

SUMMARY

Analyses to determine the chemical composition of tung fruit at different stages in its development showed that during the period of study from June 28 to November 29, 1940, the percentage of dry matter generally increased in the whole fruit, hull, outer integument, shell, and kernel. Only minor changes in actual dry weight were noted in the hull, outer integument, and shell. The major changes in dry weight of the fruits occurred in the kernels. By September 6 the kernels had attained 58.4 percent of their final weight of oil (November 1). Both the dry weight and oil content of the kernels increased from July 26 to November 1 (maturity). In general protein nitrogen, polysaccharides, and sucrose accumulated concomitantly with the oil.

Protein nitrogen, determined as the alcohol-insoluble nitrogen fraction, accumulated gradually in the kernel during the period of oil synthesis. The decrease of soluble and insoluble nitrogen in the hull and shell indicates that the proteins were probably hydrolyzed and the soluble nitrogenous products translocated to the kernel for the production of protein nitrogen.

Starch increased in the shell during the latter part of the study. The starch in the hull did not appear to have contributed to any appreciable extent to the production of storage substances in the kernel. The polysaccharides showed minor changes as compared with the other carbohydrate fractions.

The reducing sugars and starch accumulated in the kernel (mainly nucellus on the first two sampling dates) during the latter part of June and early July and then decreased rapidly to only a trace at the time of oil synthesis. This diminution indicates that these carbohydrates are utilized for the synthesis of oil and other constituents. The decrease in weight per fruit of reducing and nonreducing sugars in the hull and shell and the increase of oil, polysaccharides, protein nitrogen, and sucrose in the kernel indicate that these carbohydrates are translocated to the kernel for the production of these substances. The translocation of the soluble sugars and nitrogenous substances from the hull and shell into the kernel would not be sufficient for the production of all of the storage products. Hence, most of the sub-

stances necessary for the synthesis of these products must be brought in from other parts of the tree.

There were no significant gains in the oil content of the immature tung fruits during storage, thus indicating that sugars and other substances must be translocated into the fruit from other parts of the tree for the synthesis of oil. Prematurely abscised tung fruits had a lower oil content than mature fruits. Delayed abscission after maturity did not increase the oil content of the fruits.

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